Express Mail No. EV064840334US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#14 Plunkett 4/14/02 (NE)

Applicants	:	Jiangchun Xu et al.
Application No.	:	09/656,668
Filed	:	September 7, 2000
For	:	OVARIAN TUMOR SEQUENCES AND METHODS OF USE THEREFOR
Examiner	:	Monika B. Sheinberg Not Signed
Art Unit	:	1631
Docket No.	:	210121.484C3
Date	:	April 4, 2002

DECLARATION OF STEVE FLING Ph.D.

Commissioner for Patents
Washington, D.C. 20231

The undersigned, Dr. Steve Fling, hereby declares:

1. I am a Scientist at Corixa Corporation, the assignee of the subject application. The following experiments were carried out under my supervision.

2. Expression analysis of ovarian cancer-associated sequence SEQ ID NO: 198 or O590S was performed by Real-Time PCR. Real Time PCR (*e.g.*, Gibson et al., *Genome Research* 6:995-1001, 1996; Heid et al., *Genome Research* 6:986-994, 1996) is a technique that evaluates the level of PCR product accumulation during amplification. This technique permits quantitative evaluation of mRNA levels in multiple samples. Briefly, mRNA was extracted from tumor and normal tissue and cDNA was prepared using standard techniques. Real Time PCR was performed using a Perkin Elmer/Applied Biosystems (Foster City, CA) 7700 Prism instrument. Matching primers and fluorescent probes were designed for O590S (SEQ ID NO:198) using the primer express program provided by Perkin Elmer/Applied Biosystems (Foster City, CA). Optimal concentrations of primers and probes were determined and control (*e.g.*, β-actin) primers and probes were obtained commercially from Perkin Elmer/Applied Biosystems (Foster

City, CA). To quantitate the amount of O590S specific mRNA in a sample, a standard curve was generated using a plasmid containing O590S. A standard curve was generated using the Ct values determined in the Real Time PCR, which were related to the initial cDNA concentration used in the assay. Standard dilutions ranging from 10^1-10^6 copies of the gene of interest were generally sufficient. In addition, a standard curve was generated for the control sequence. This permits standardization of initial RNA content of a tissue sample to the amount of control for comparison purposes.

Real Time PCR analysis demonstrated that SEQ ID NO:198, clone 57886 or O590S, was shown to be over-expressed in over 65% of ovarian tumor samples tested, 100% of tumor samples derived from SCID mice, and 100% of ovarian tumor cell lines tested, when compared to an extensive panel of normal tissue. Little or no expression was observed in normal esophagus, spinal cord, bladder, colon, liver, PBMC (activated or resting), lung, skin, small intestine, stomach, skeletal muscle, pancreas, dendritic cells, heart, spleen bone marrow, thyroid, trachea, thymus, bronchia, cerebellum, ureter, uterus and peritoneum epithelium. Some low level expression was observed in normal breast, brain, bone, kidney, adrenal gland and salivary gland, with the expression levels in these normal tissues generally at least several fold lower than the levels observed in ovary tumors over-expressing O590S.

3. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

_____ _____

Steve Fling, Ph.D. Date

C:\NrPortbl\iManage\HELENM\259721_1.DOC